Toxic Effects of a Whole-body Inhalation Sarin (GB) Vapor Exposure in the Gottingen Minipig

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Joint Services Conference
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U.S. Army RDECOM

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Benefits of the minipig

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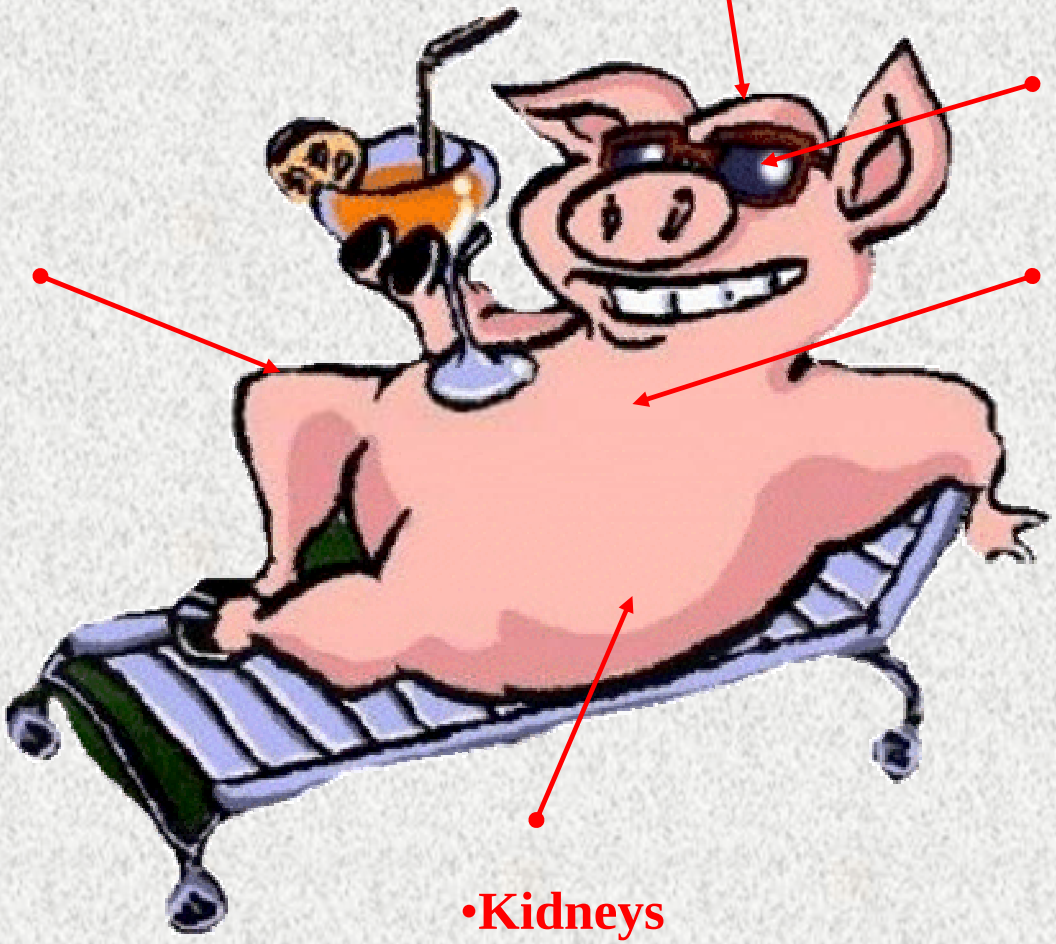

- Brain
- Visual system
- Cardiovascular/ Pulmonary system
- Dermis
- Kidneys

Gottingen Minipigs

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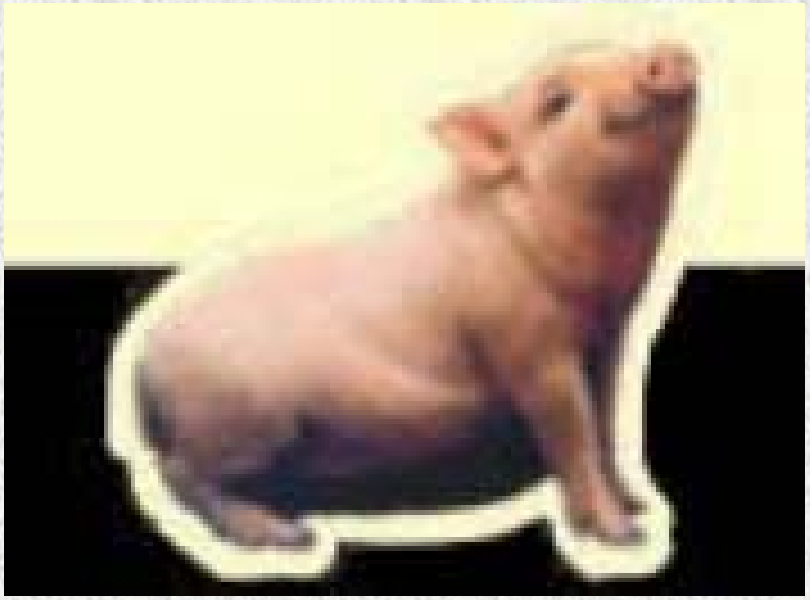

- Well defined genetic background and health status
- Small size at sexual maturity
  - Males 7-9 kg (3-4 months)
  - Females 9-11 kg (4-5 months)

Experimental preparations

- Exposure chambers modified/set-up for whole-body exposure of minipigs.
- Gottingen (Ellegaard) minipigs (10-15 Kg)
  - Pigs surgically prepared with jugular cannulas for serial blood sampling during exposure
  - IR/digital video camera and digital image capture system developed to monitor and calculate pupil size
    - Real-time monitoring of first noticeable effect (FNE) during exposure

Vapor GB generation chamber

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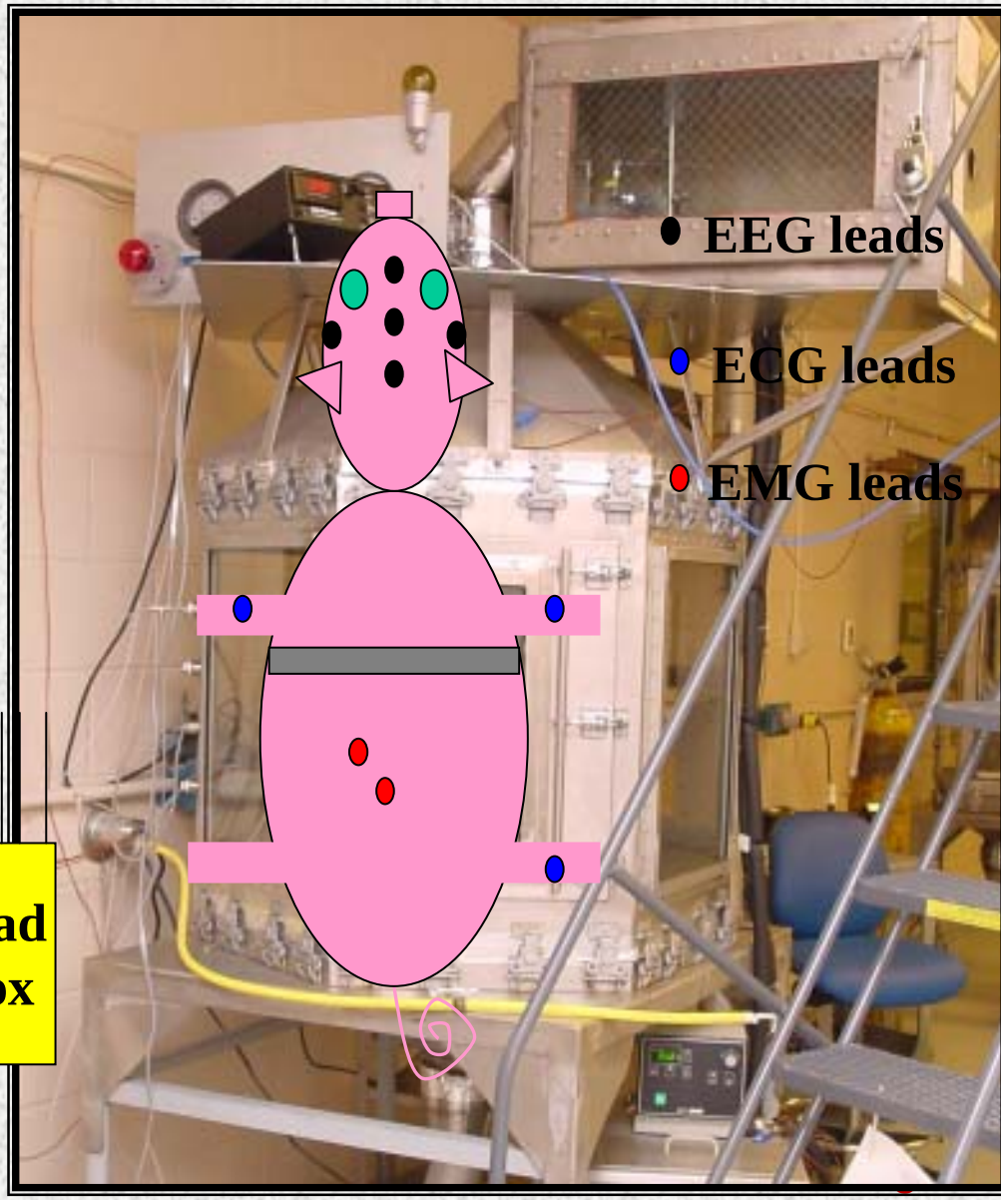
- Pig placed in sling
- Respiratory belt, ECG leads and EEG leads attached to pig and leading to Bio-logic headbox.
- 1000 L dynamic airflow chamber
- GB generation system contained in glove box
  - Vapor Sampling / Analysis
- Jugular catheter passed through ports
- IR images of pupil taken through plexiglass



Exposure

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- 5.35 mg/m^3 vapor GB for 10 minutes (Ct=53.5 mg.min/m^3)
- Infrared images captures continuously
- Blood samples collected every 2 minutes during exposure
- Electrophysiology system collects signals throughout exposure
- A score sheet is used to rate pigs on activity and onset/progression of signs

Data collected

- Miosis onset, Time-to-maximum miosis
- RBC and plasma cholinesterase activity
- Internal GB (GB regeneration assay)
- Blood Chemistry
  - Lactate, glucose, creatinine, hematocrit, hemoglobin, Sodium, Potassium, pH, HCO3, SaO2, PO2, PCO2, TCO2, base excess
- Electrocardiogram
  - Heart rate (R-R interval), PR interval, QT interval, QTc interval, ST interval, QRS interval
- EEG, EMG, airflow, eye movements, respiratory rate,
- Distribution of GB in tissue

➢First and only studies looking at the global toxicological affects of a whole-body nerve agent vapor exposure in real-time

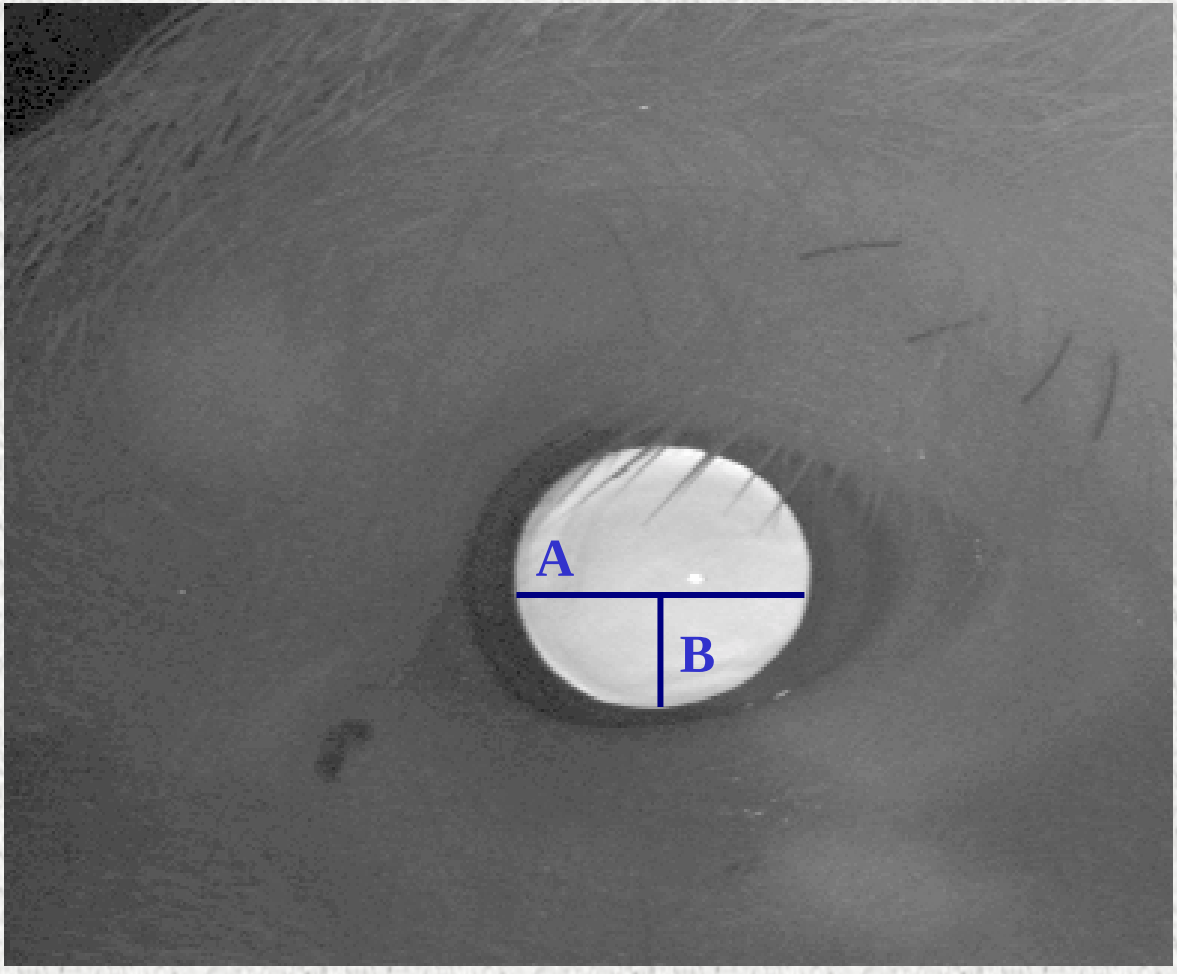
➢Large scale operation that requires the help and expertise of many people

Data collection methods / Biological endpoints

- Infrared camera
  - Allows images under dim light conditions
  - Uses pupil areas to plot time-to-miosis
- Insertion of external jugular catheter
  - Serial blood samples during “real-time” exposure
    - RBC and plasma cholinesterase
    - GB regeneration assay
    - Blood Chemistry (iStat)
- Physiological monitoring (Bio-logic, Inc.)
  - EEG, EKG, EMG, airflow, eye movements, respiratory rate, heart rate, SaO_2
- Necropsy
  - Tissue GB levels

Infrared pupillometry

Baseline



- Infrared light reflects off of the retina
- Pupil area = A*B*π

60 minute GB exposure- 0.047 mg/m^3

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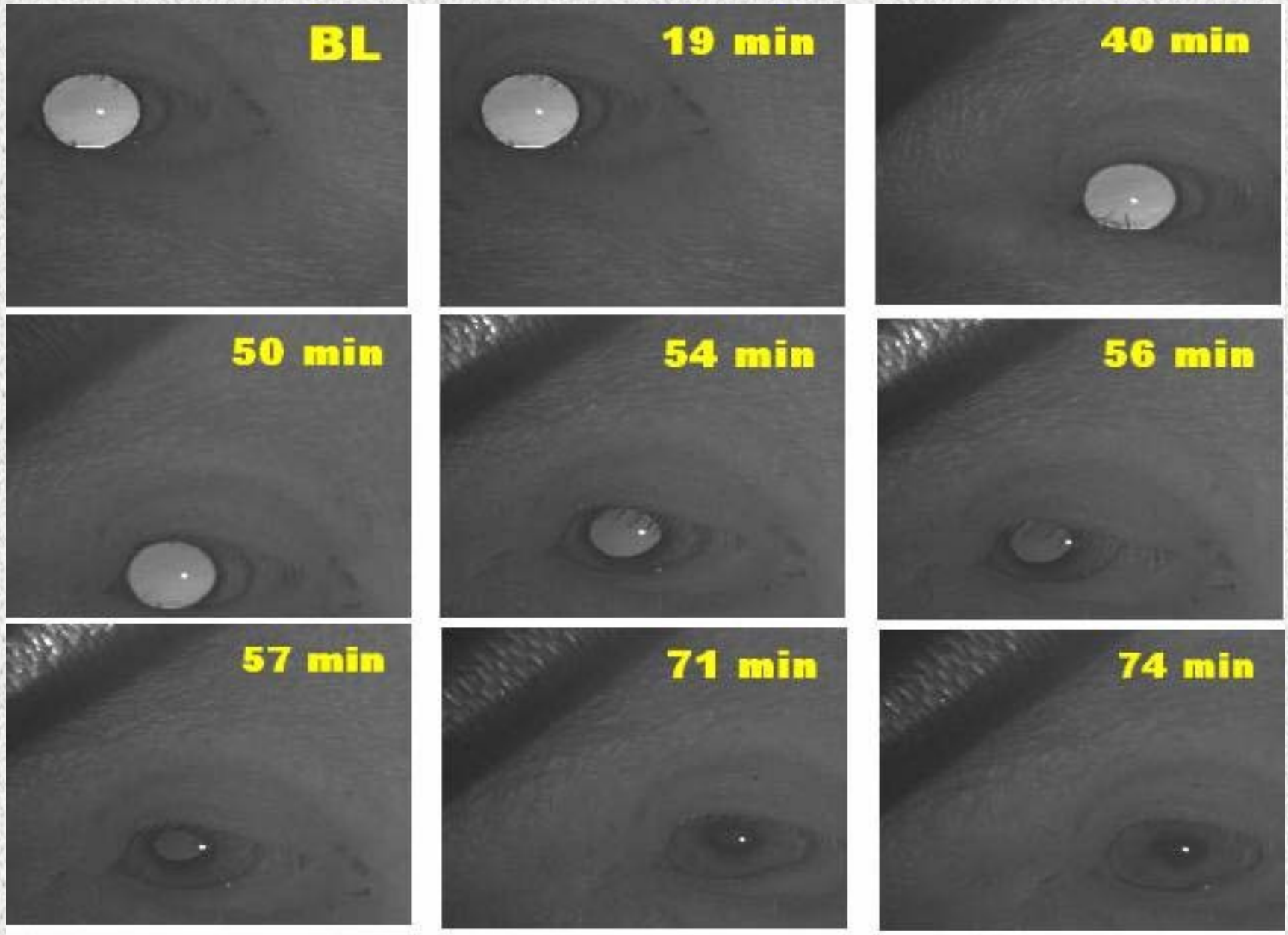

Pupil Constriction

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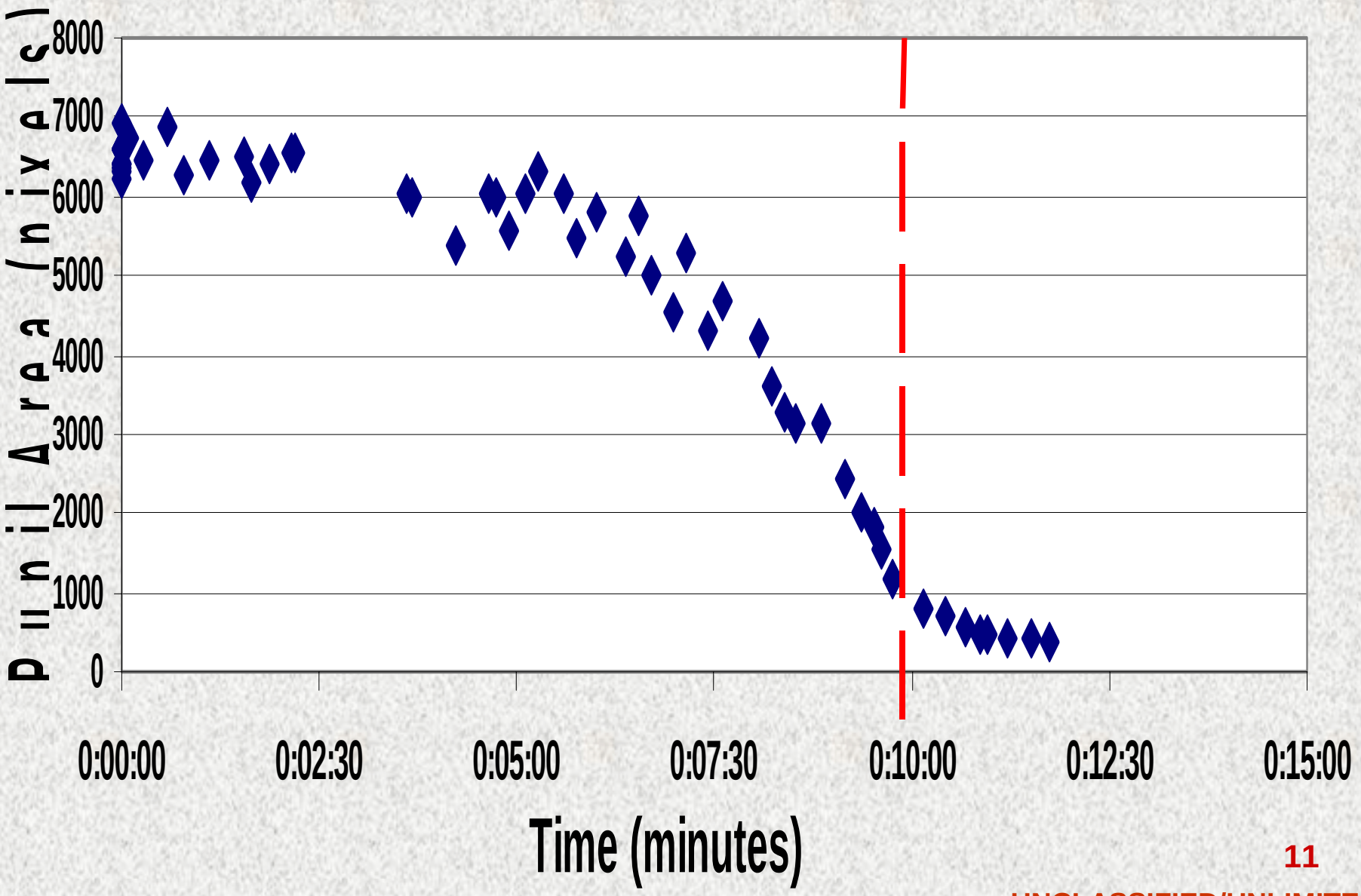

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Data collection methods / Biological endpoints

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Cholinesterase Activity

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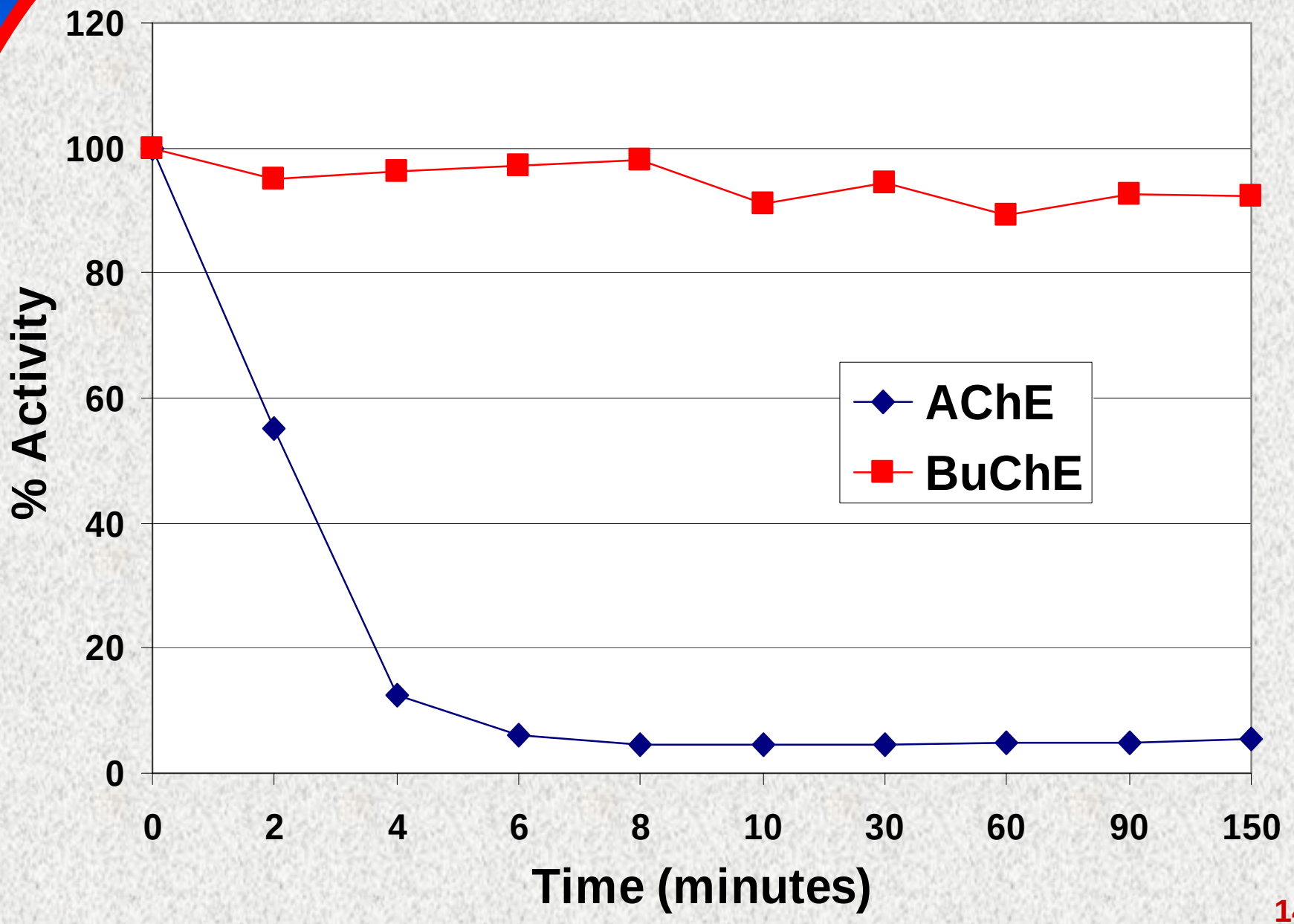

Data collection methods / Biological endpoints

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    - GB regeneration assay → M.Jakubowski
    - Blood Chemistry (iStat)
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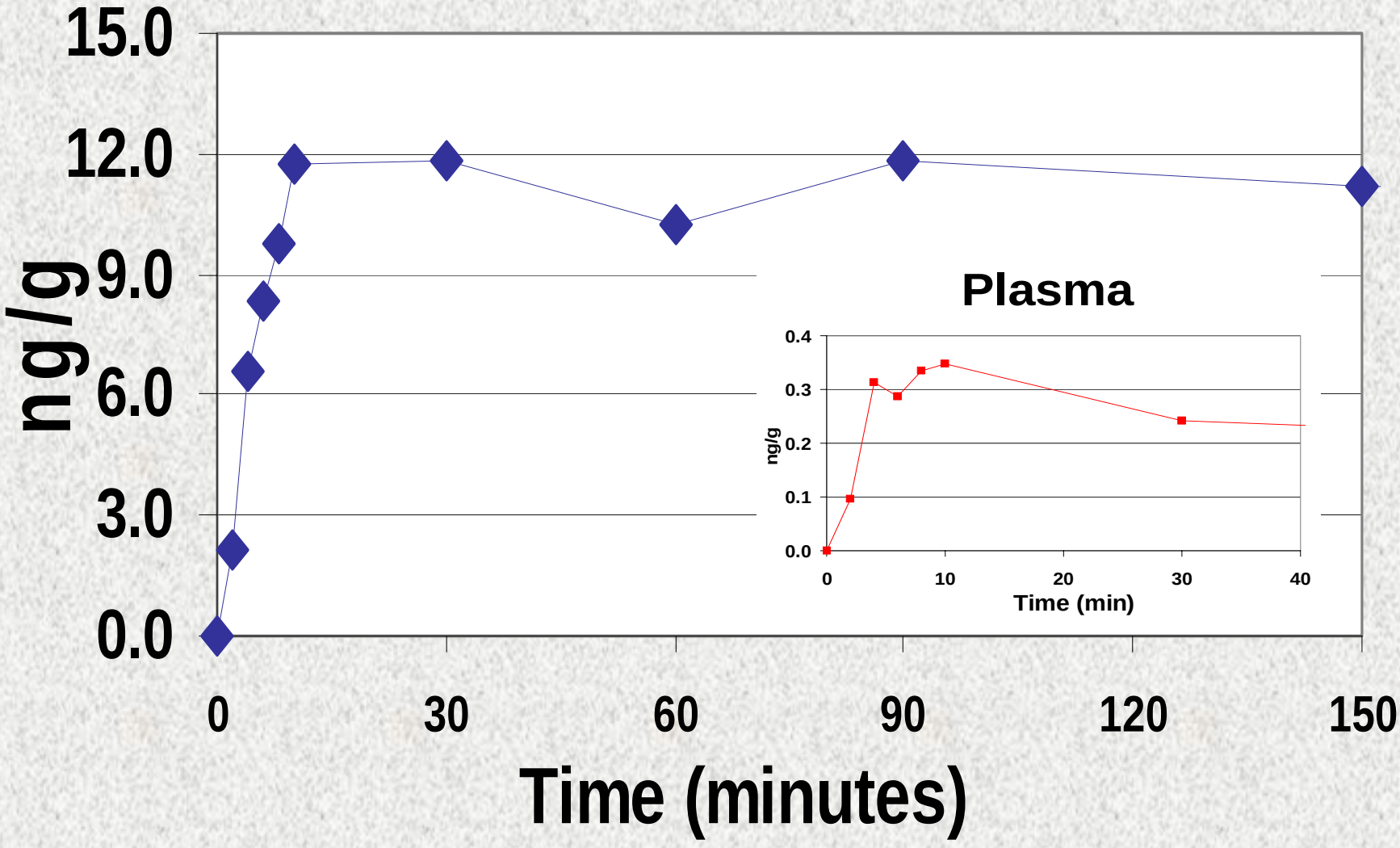
- Necropsy
  - Tissue GB levels
  - Changes in gene transcription

RBC REGENERATED GB



Data collection methods / Biological endpoints

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    - GB regeneration assay
    - Blood Chemistry (iStat) → J. Forster
- Physiological monitoring (Bio-logic, Inc.)
  - EEG, EKG, EMG, airflow, respiratory rate, heart rate, SaO_2
- Necropsy
  - Tissue GB levels
  - Changes in gene transcription

iStat blood analyzer

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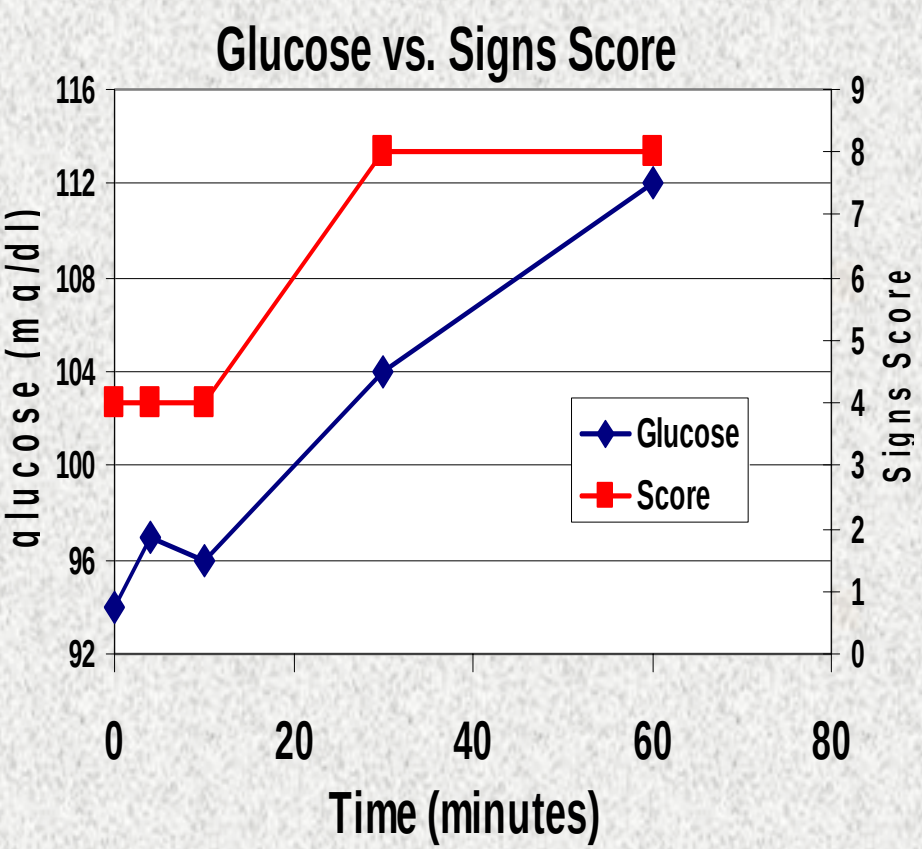

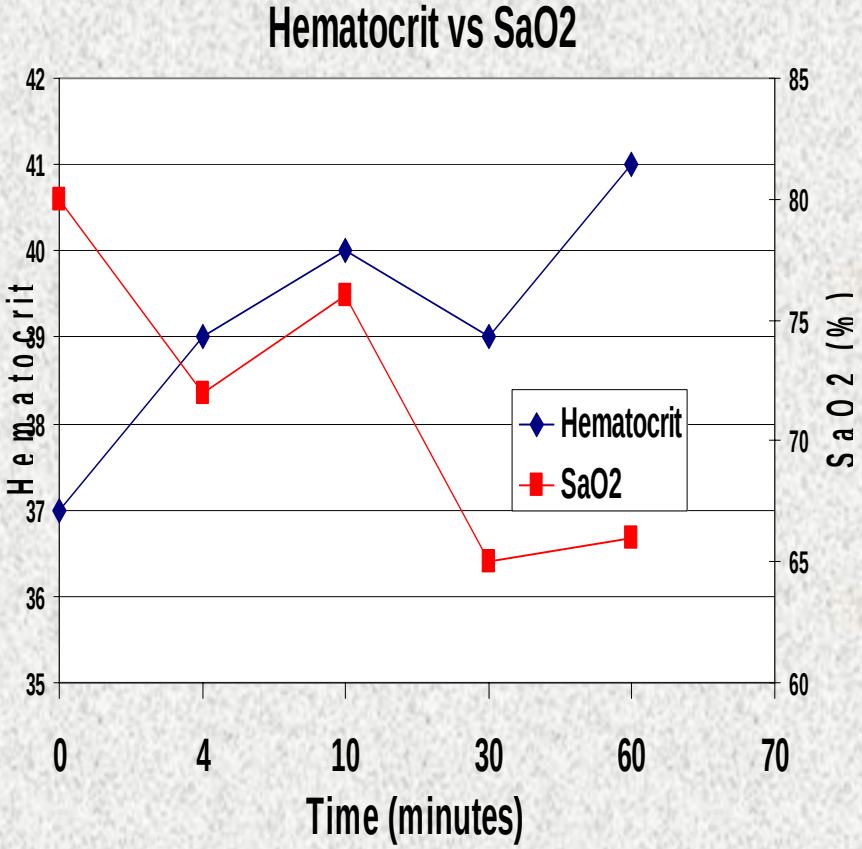

Also can assess creatinine, lactate, pH, hemoglobin, Na, K, TCO_2, PCO_2, PO_2, HCO_3 and Base excess

Data collection methods / Biological endpoints

- Infrared camera
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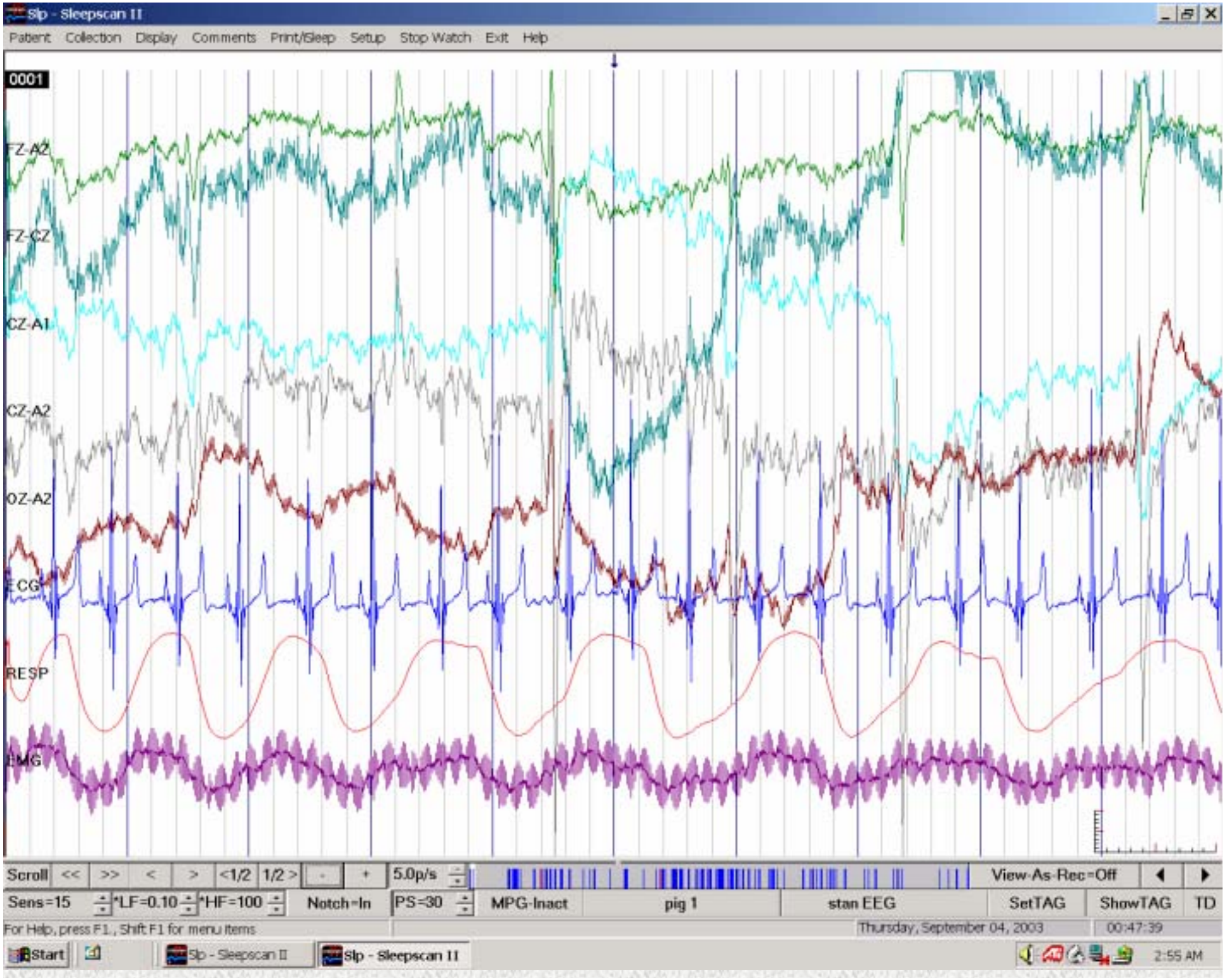
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FZ-A2
FZ-CZ
CZ-A1
CZ-A2
OZ-A2
ECG
RESP
EMG
Scroll << >> < > <1/2 1/2 > - + 5.0p/s
View-As-Rec=Off
Sens=15 *LF=0.10 *HF=100 Notch=In PS=30 MPG-Inact pig 1 stan EEG SetTAG ShowTAG TD
For Help, press F1, Shift F1 for menu items
Thursday, September 04, 2003
00:47:39
Start
Slp - Sleepscan II
Slp - Sleepscan II
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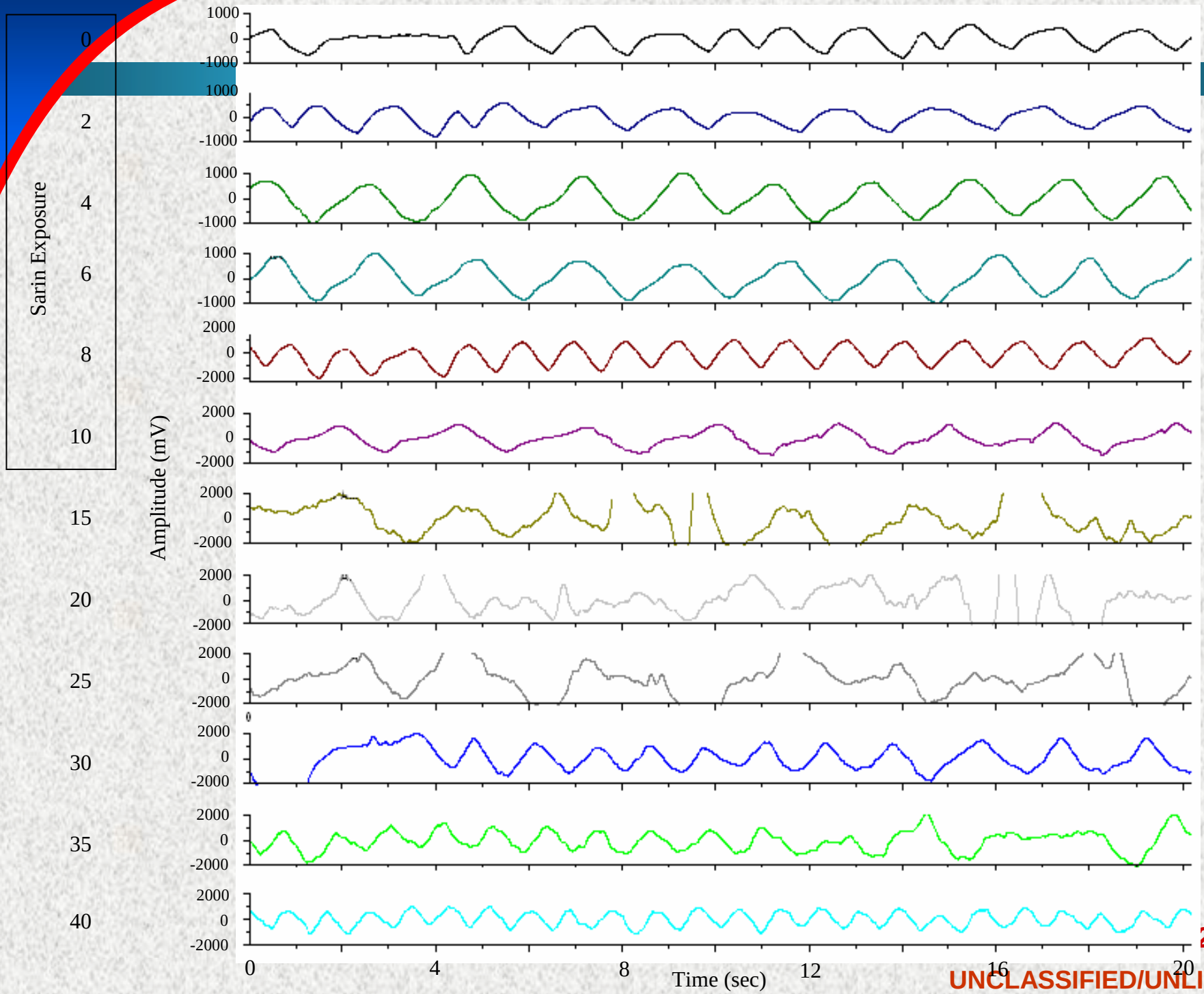
Time (min)
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0
2
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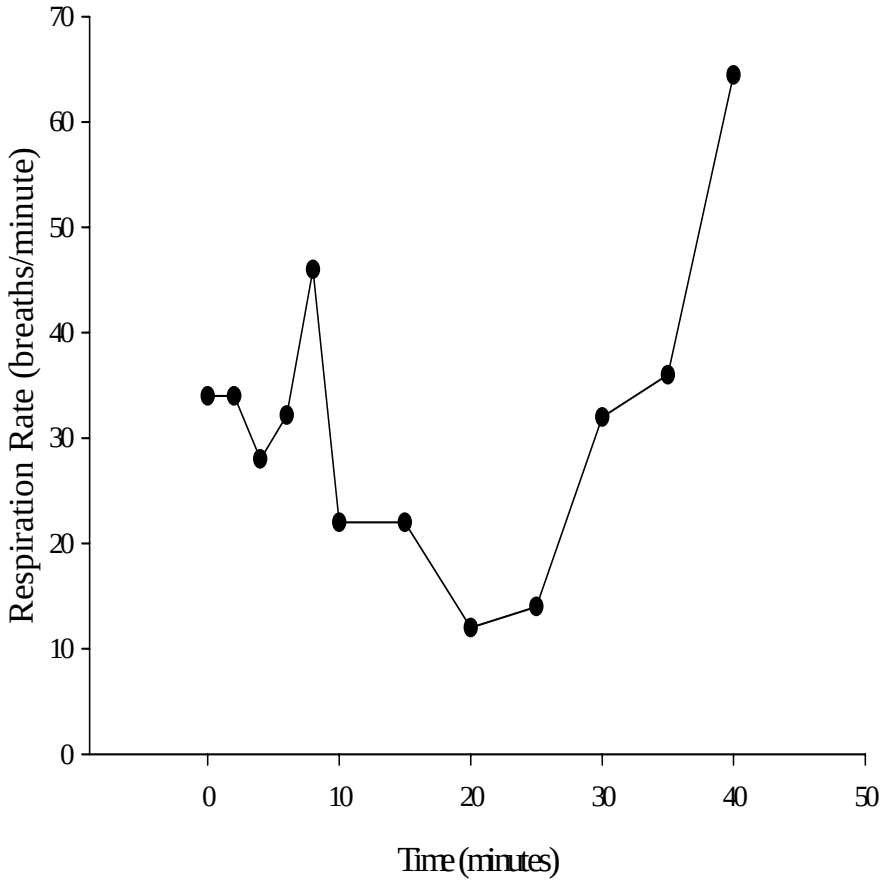
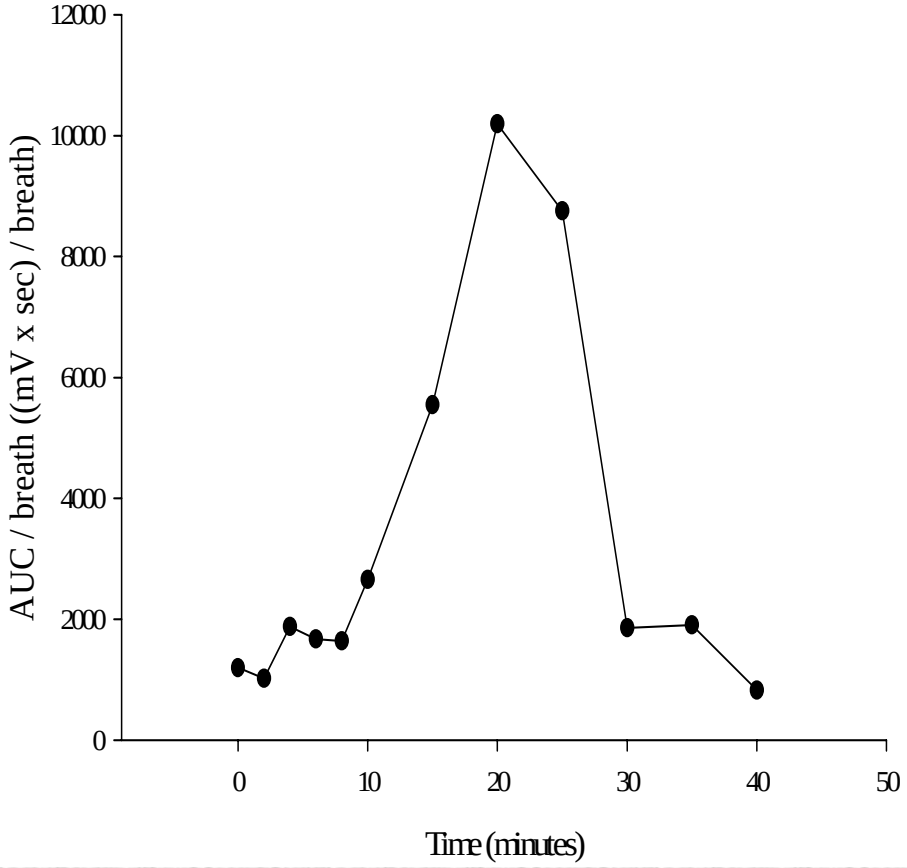
Time (sec)



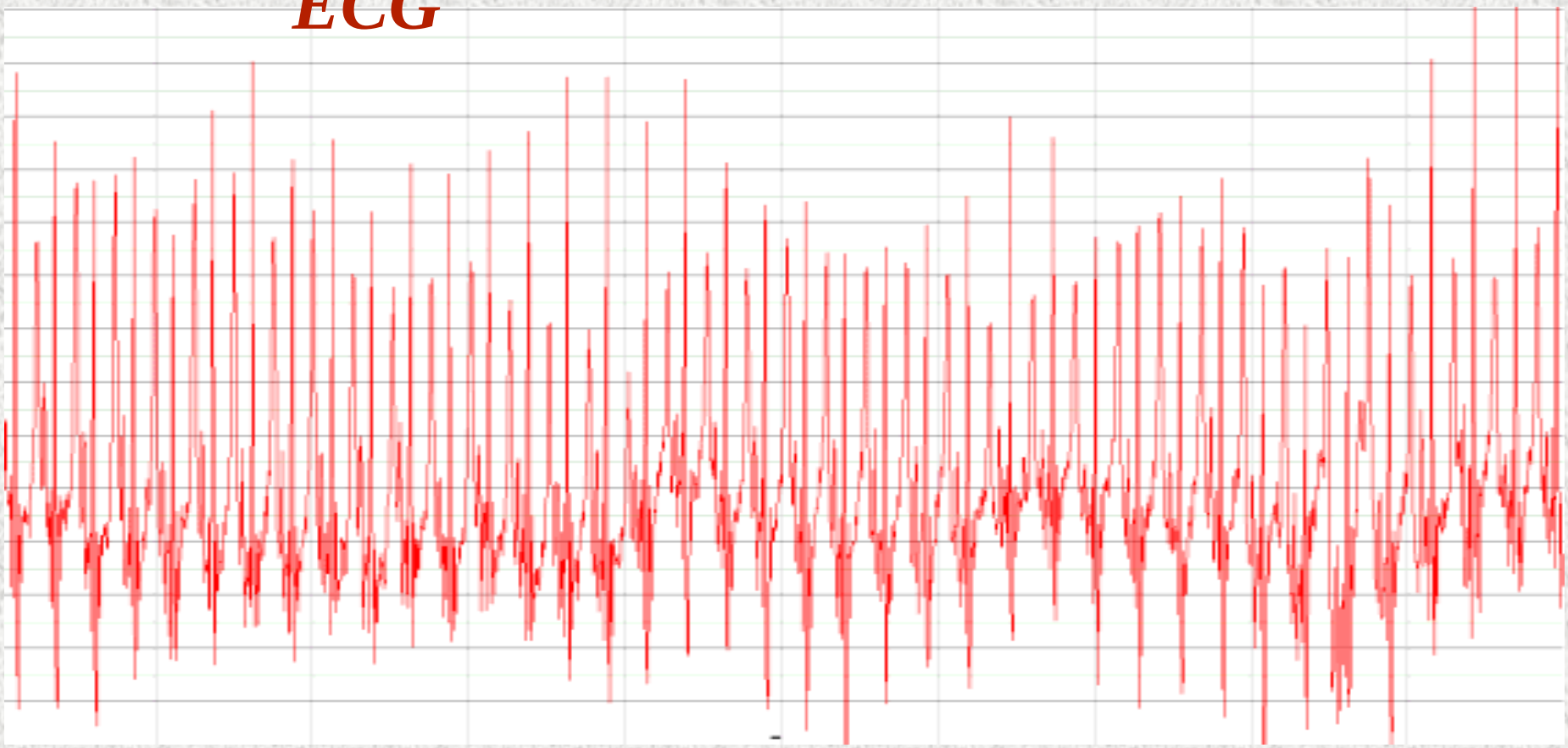


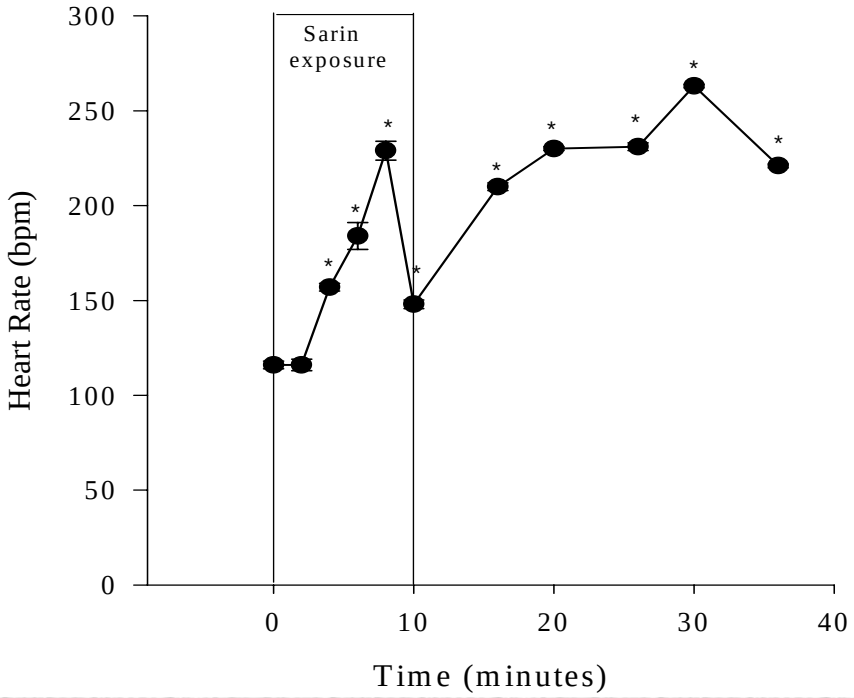
- **Respiratory rate is depressed**
- **Breaths become deep, long and irregular**

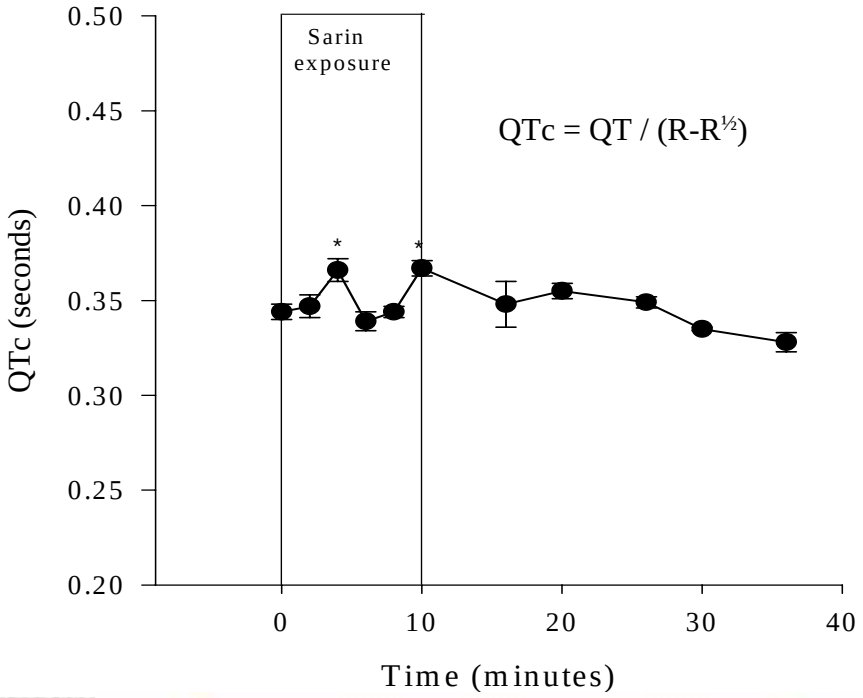
ECG analysis

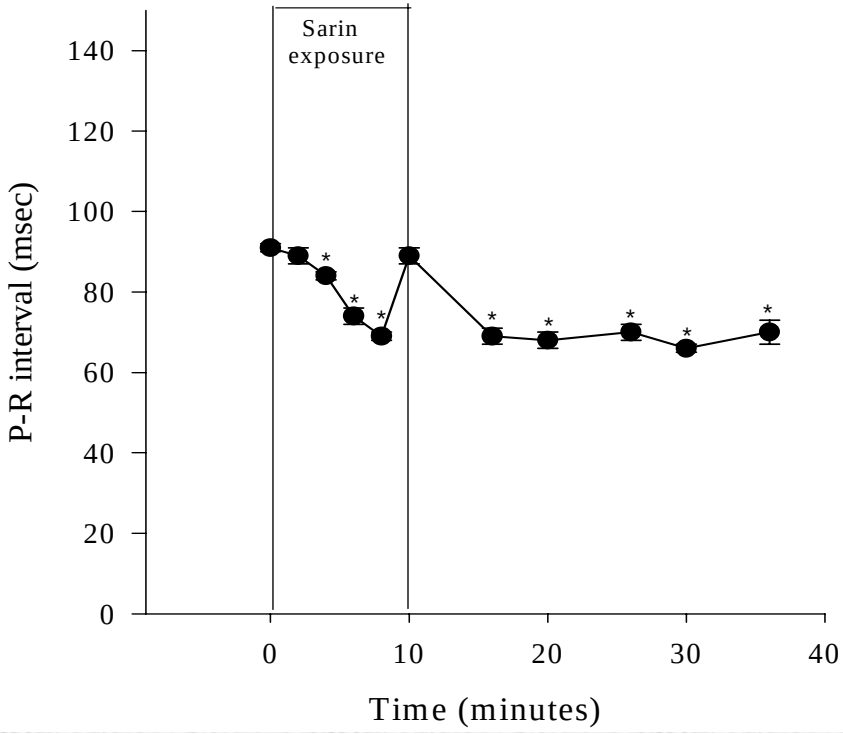
- ECG signals recorded on Biologic Inc. system
- Standard Lead II configuration
  - Historical data available
- Exposure broken down into 2 minute segments
  - Approximately 30 second epochs during each segment analyzed
- Data analyzed using Dataquest ART and Statview
  - Heart rate (R-R interval)
  - PR interval
  - QT interval
  - QTc interval
  - ST interval
  - QRS interval

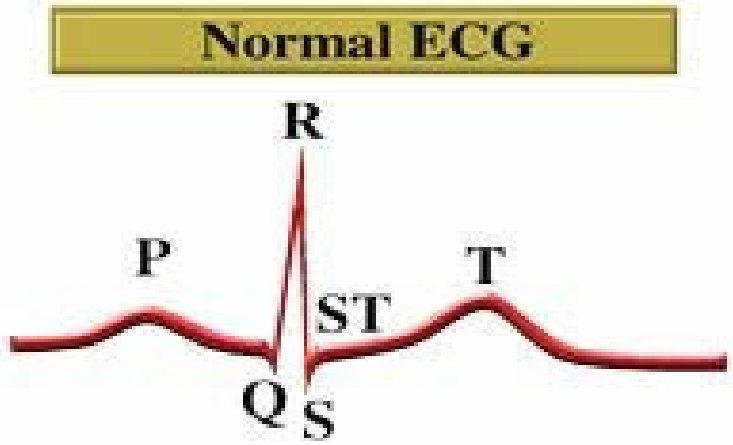
ECG







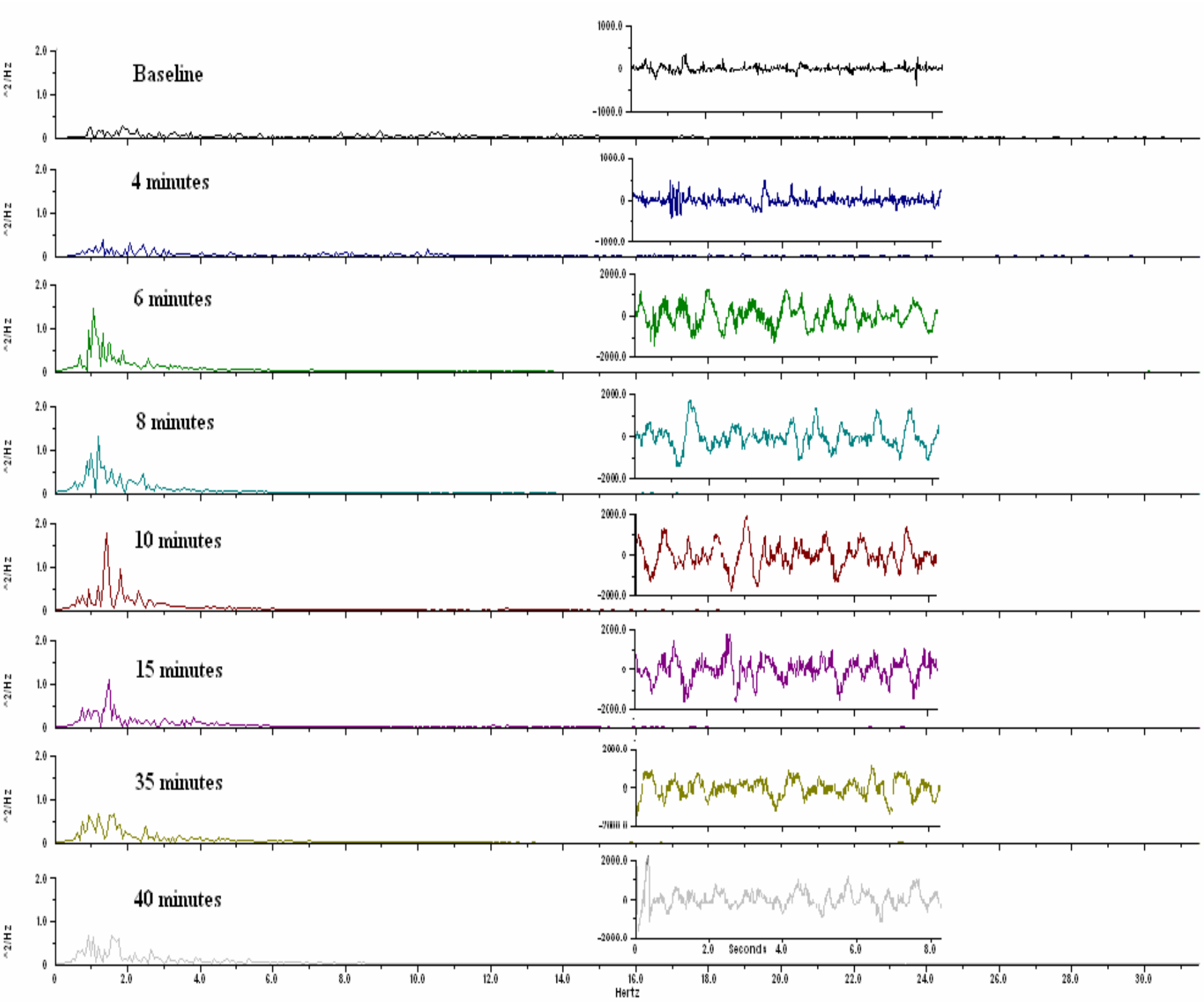




Suggests delayed ventricular repolarization

EEG analysis

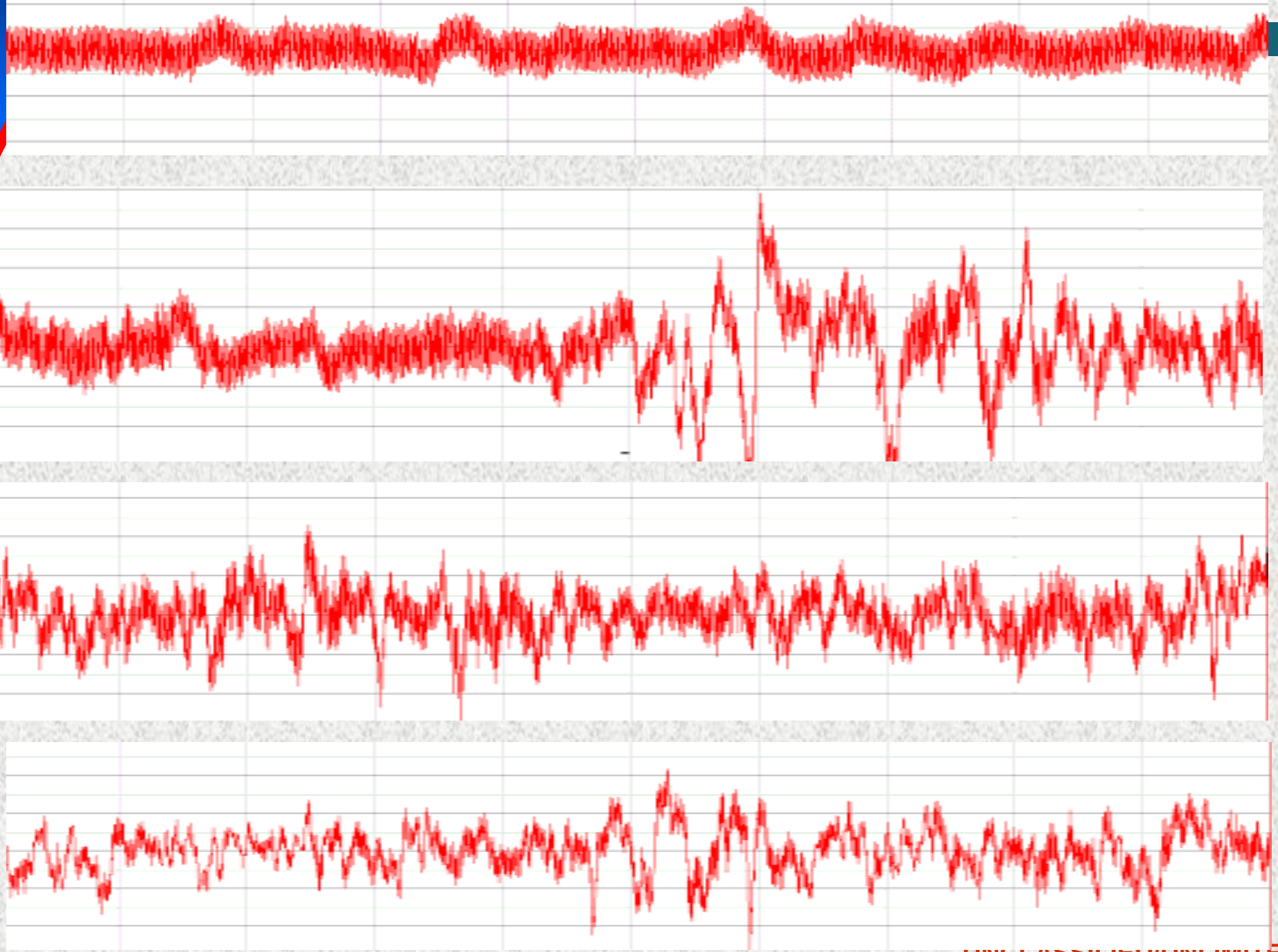
- EEG signals recorded on Biologic Inc. system
- Exposure broken down into 2 minute segments
  - Approximately 30 second epochs during each segment analyzed
- Power spectral density analysis using Dataquest ART
  - Bandpass filters: 1-35 Hz


Baseline
4 minutes
6 minutes
8 minutes
10 minutes
15 minutes
35 minutes
40 minutes
^2/Hz
Hertz
Seconds

EMG analysis

- EMG signals recorded on Biologic Inc. system
- Exposure broken down into 2 minute segments
  - Approximately 30 second epochs during each segment analyzed
- Power spectral density analysis using Dataquest ART

EMG



EMG power spectral analysis

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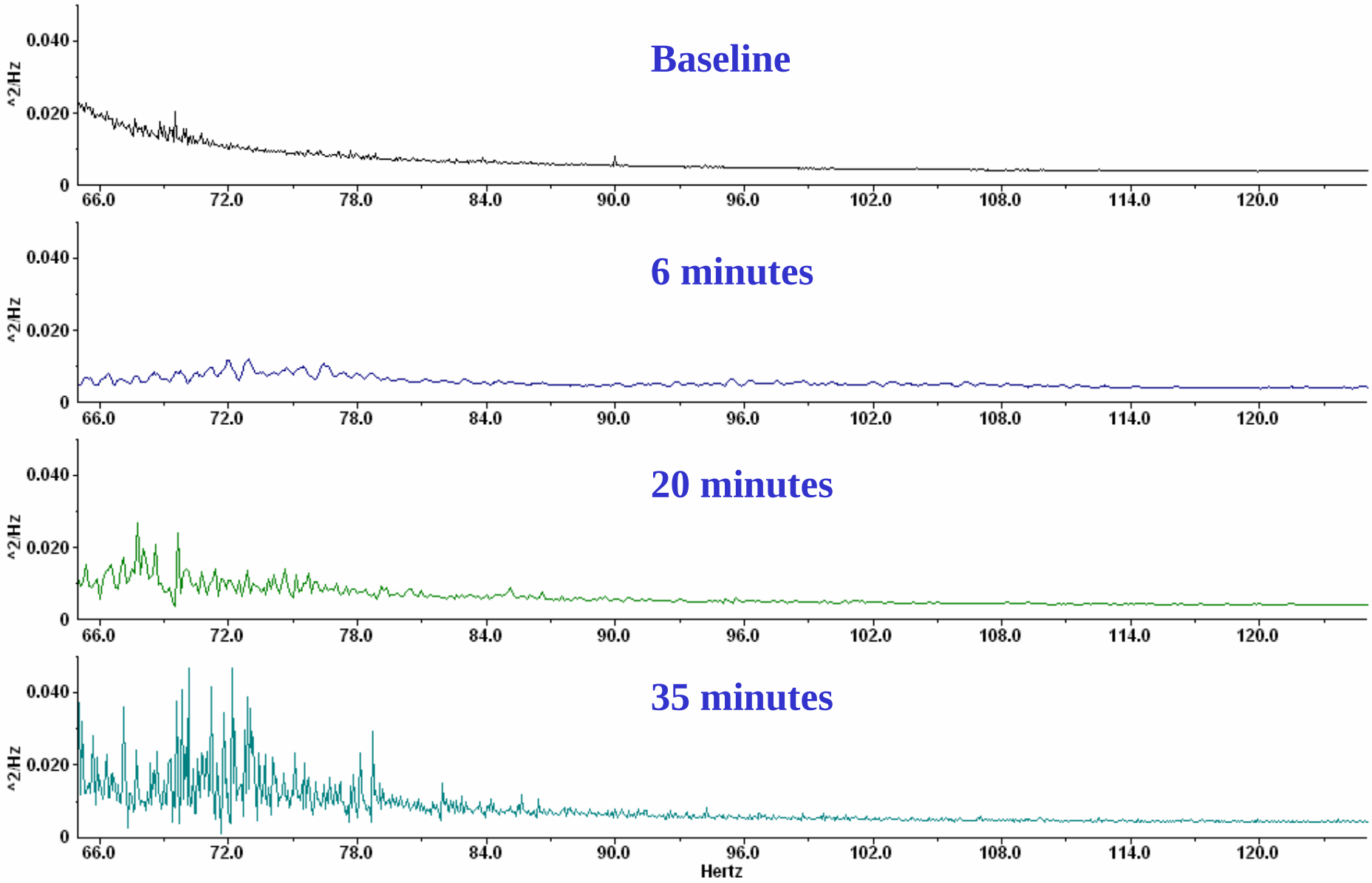

Data collection methods / Biological endpoints

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- Infrared camera
  - Allows images under dim light conditions
  - Uses pupil areas to plot time-to-miosis
  - Calculation of EC_{50} (miosis)
- Insertion of external jugular catheter
  - Serial blood samples during “real-time” exposure
    - RBC and plasma cholinesterase
    - GB regeneration assay
    - Blood Chemistry (iStat)
- Physiological monitoring (Bio-logic, Inc.)
  - EEG, EKG, EMG, airflow, respiratory rate, heart rate, SaO_2
- Necropsy
  - Tissue GB levels  M. Jakubowski

GB Tissue Distribution

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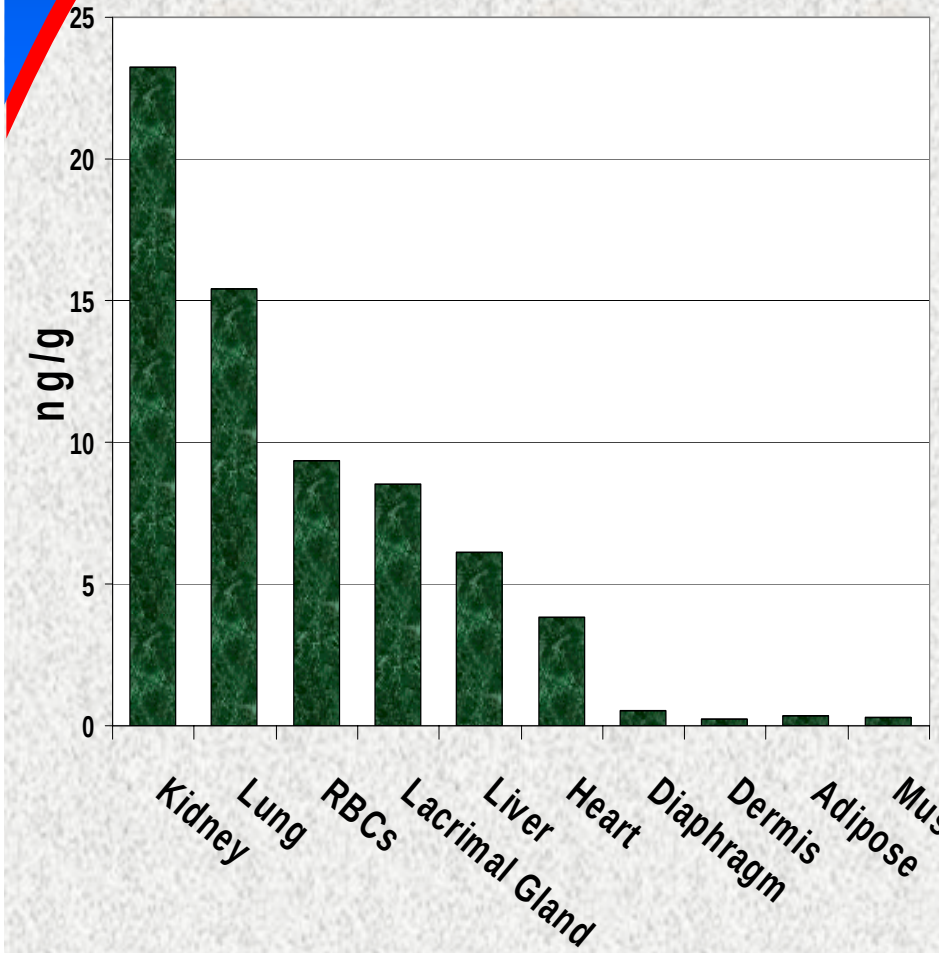

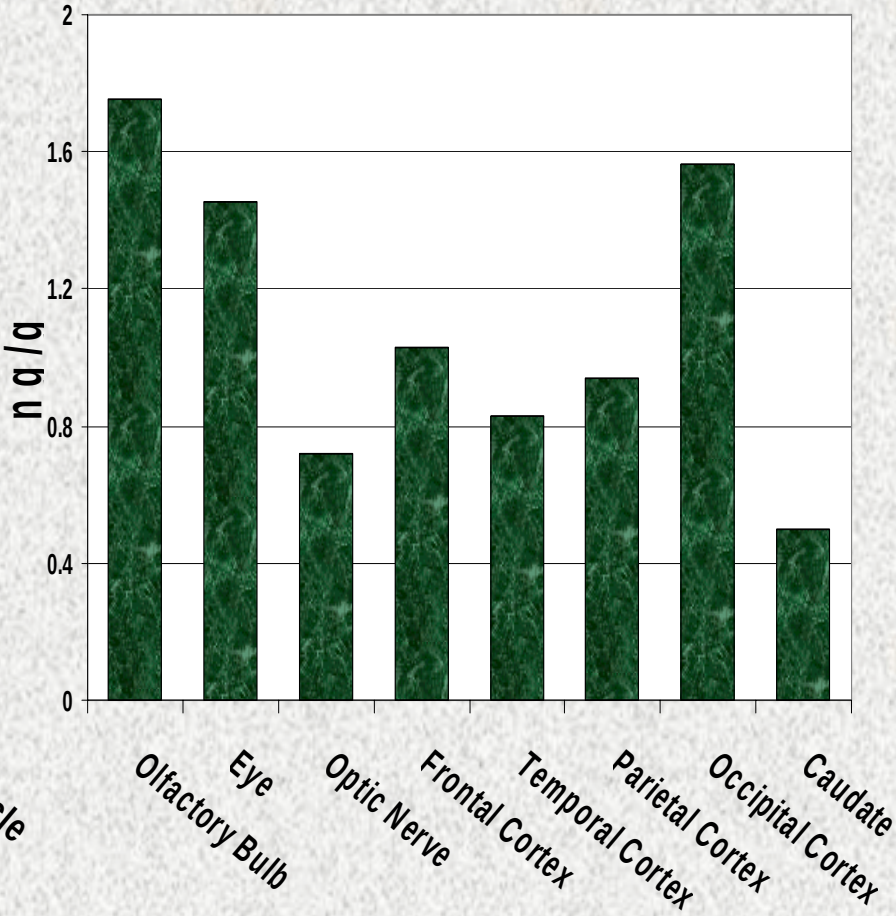

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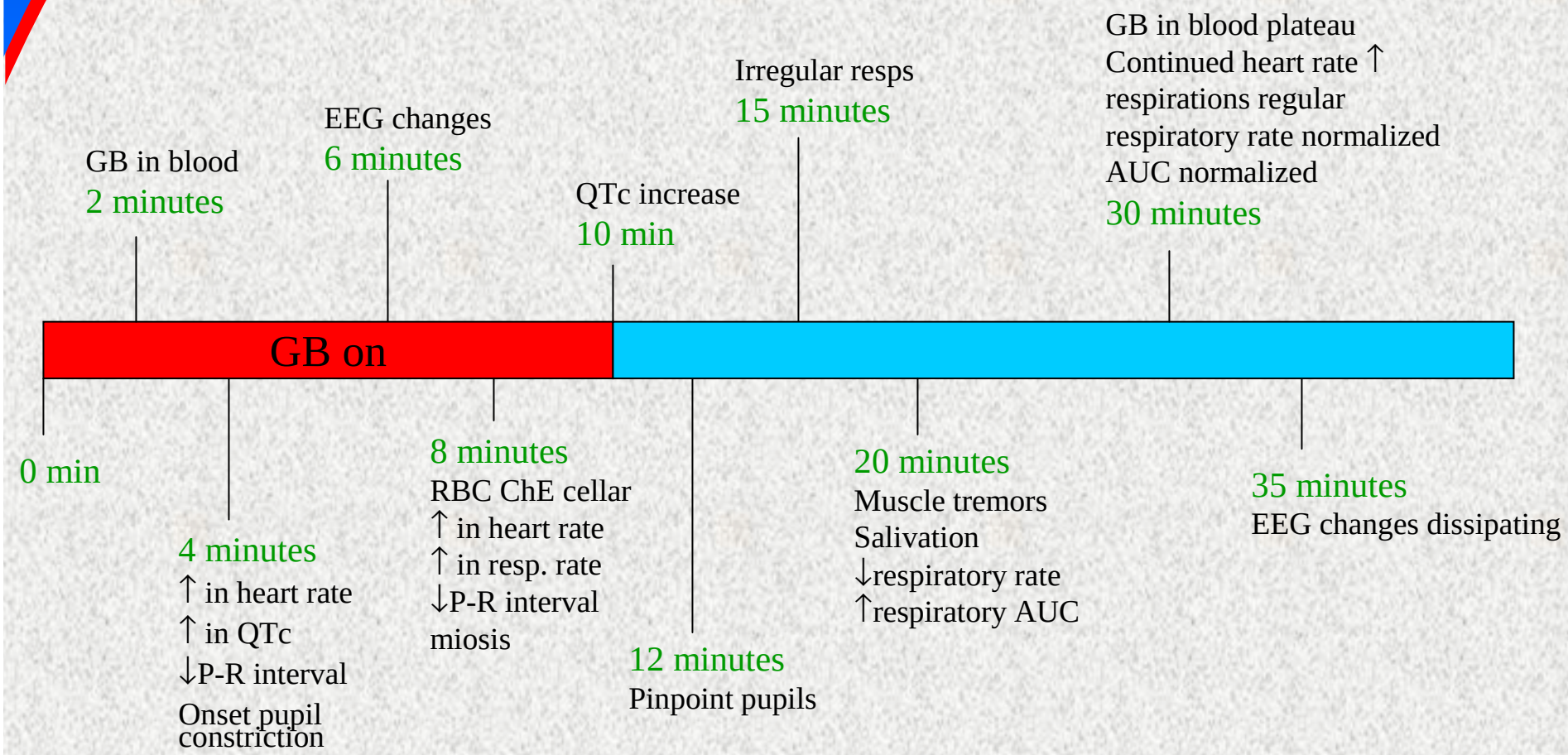

Thank You

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- Jennifer Edwards
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- Ron Evans
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- Bernardita Gaviola
- David McCaskey
- Jennifer Sekowski
- Dennis Johnson


"That's all Folks!"
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Low Level Operational Toxicology

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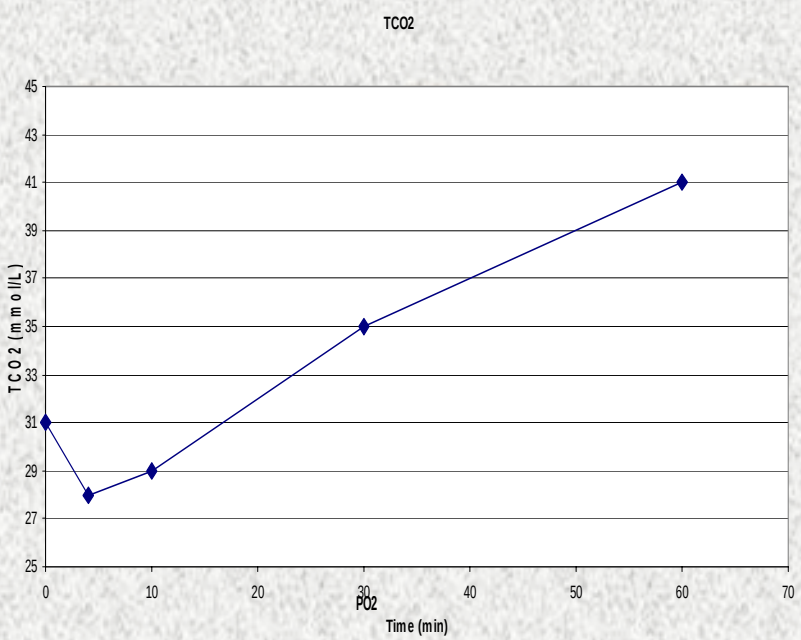

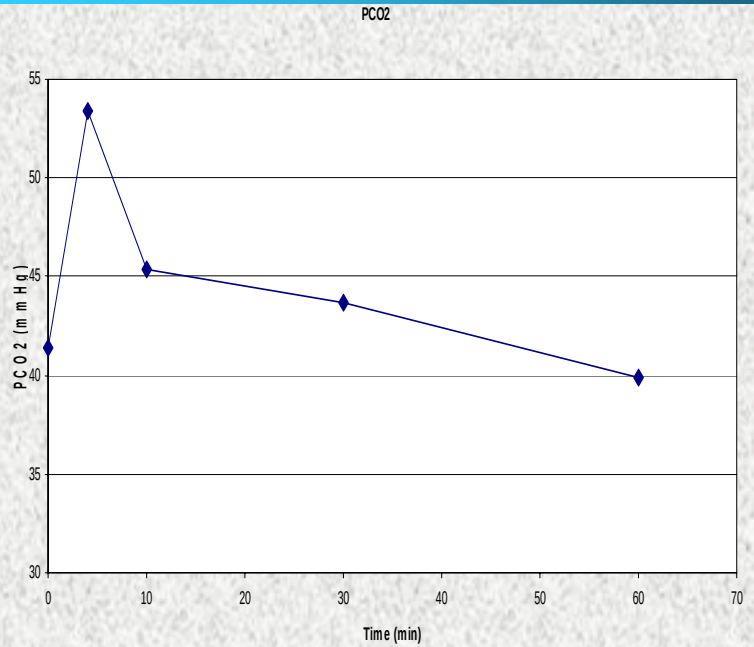

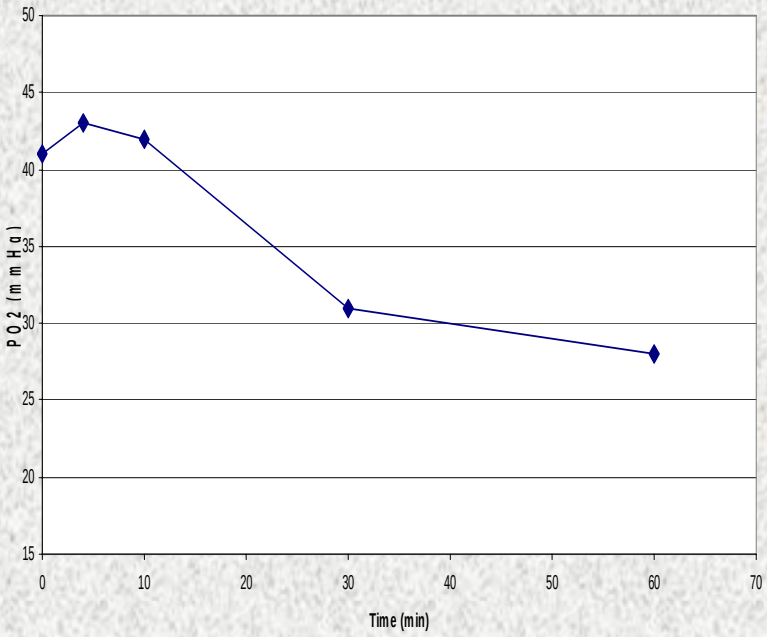

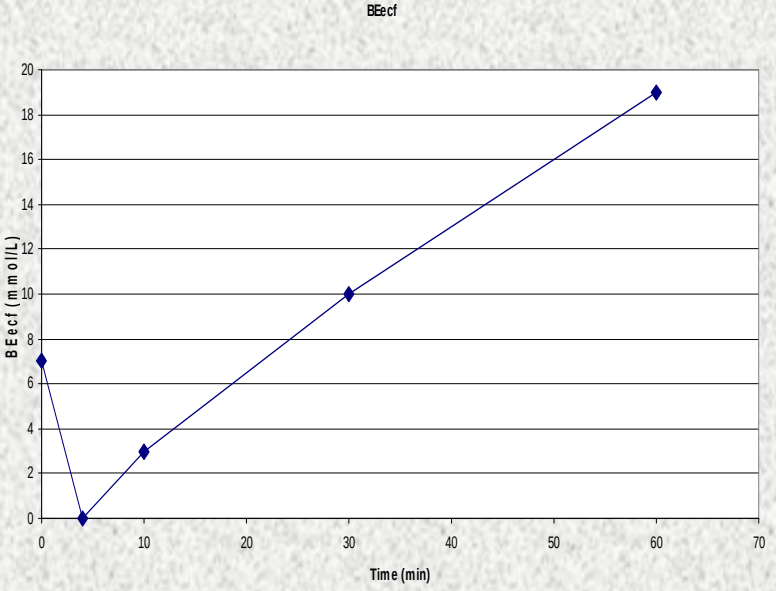

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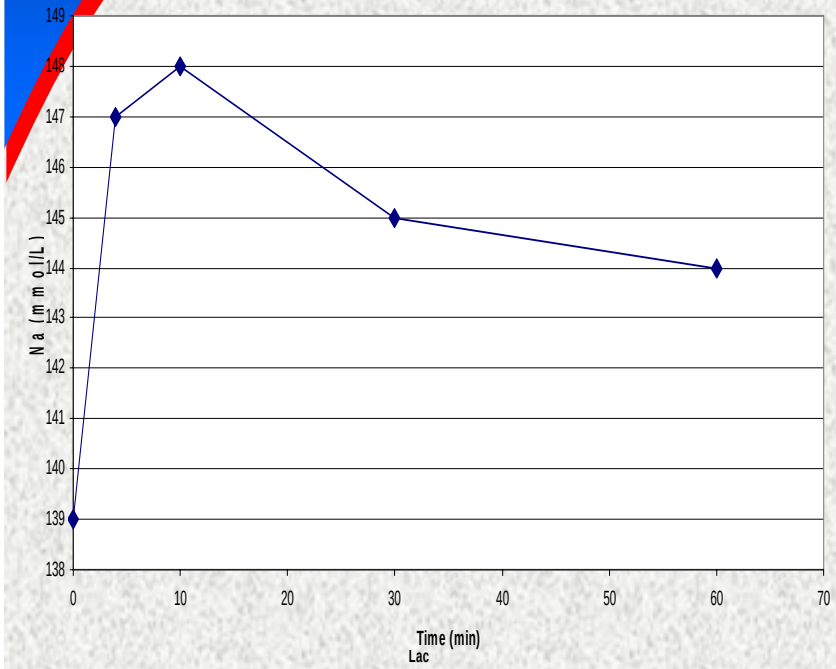
Na



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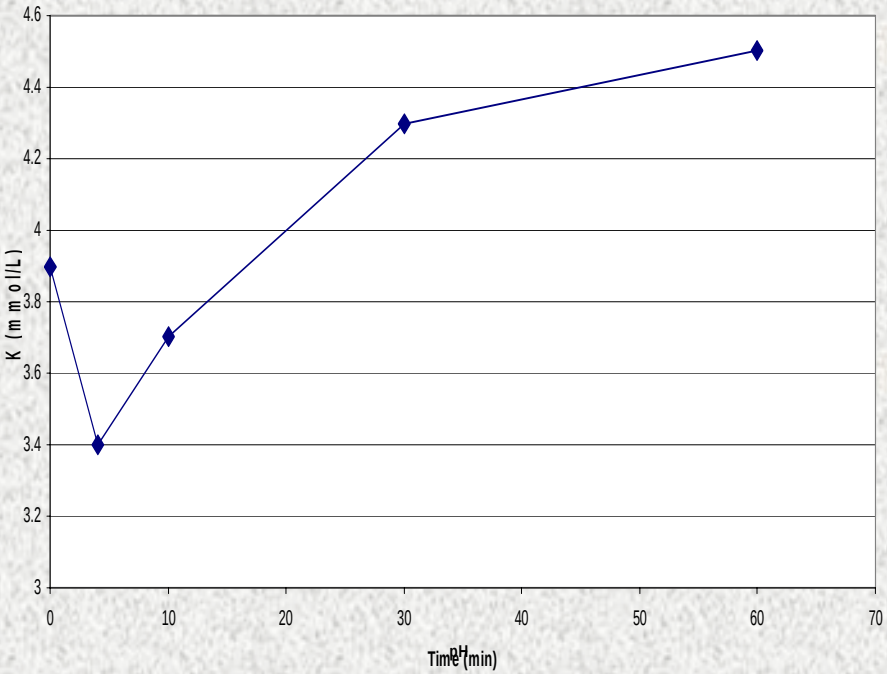

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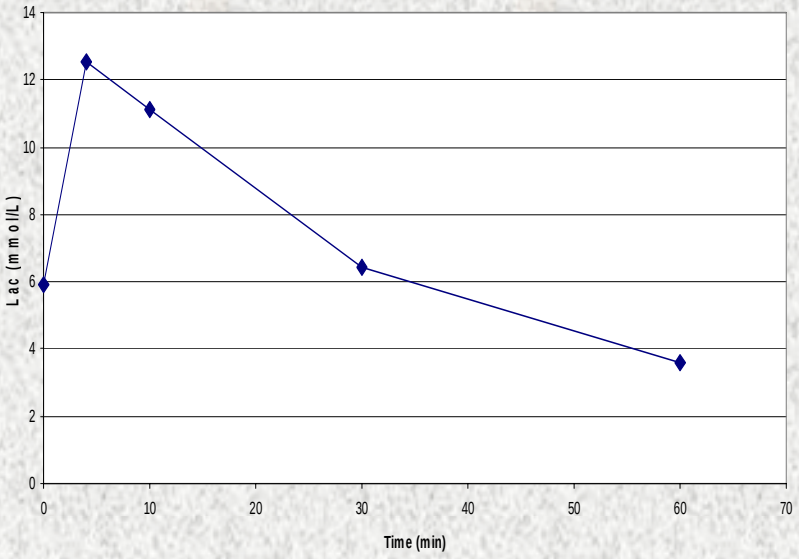
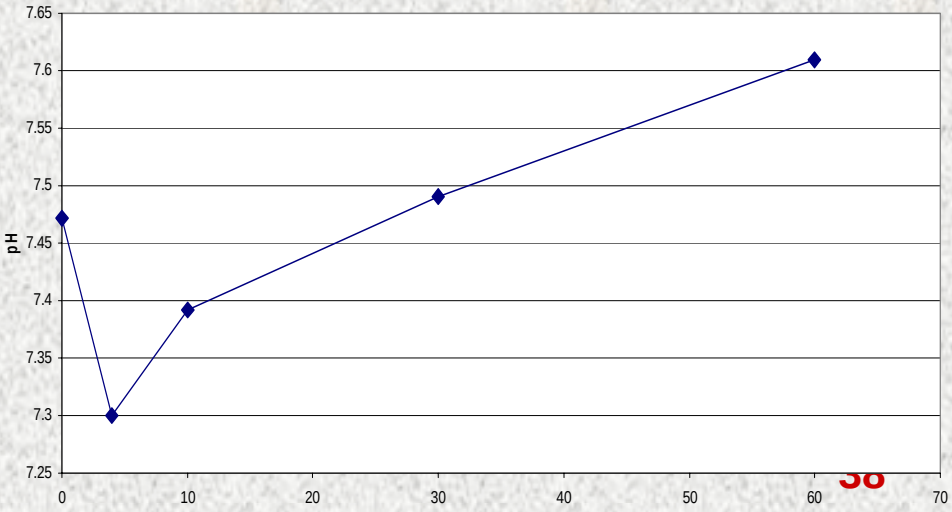


pH



Effect of Perfusion on Tissue Levels

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